JGI Microbial Single Cell Program Single Cell Data Decontamination

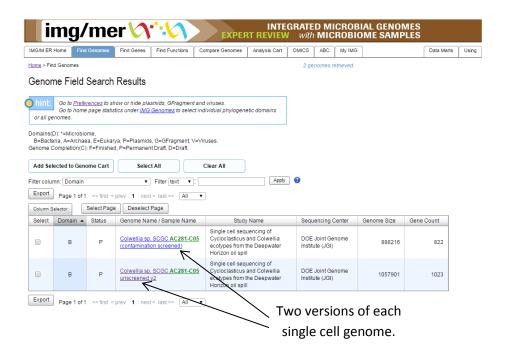
Despite our best efforts, it is possible that there are some contigs in your single cell genome that are from contaminant organisms. In addition to external contaminants, there may be contaminants from within your particular sample in the form of free DNA that made it into the well along with your single cell. All single cell genomes sequenced at the JGI go through an automated contaminant removal pipeline. In order to prevent contaminant data from propagating in the databases to cause errors in the annotation of future genomes/metagenomes, the automated system is tuned to be aggressive in its contaminant removal. This means that it is likely that some contigs that were truly from your genome have been removed. Because these contigs that were removed may be of interest to you, we provide two versions of each single cell genome: a screened version that has gone through our automated pipeline and is added to the databases for use in annotation of future genomes/metagenomes, and an unscreened version that contains all the contigs, but is not part of the databases used for annotation. If you do not want to rely on the aggressively cleaned version that we provide, you may do a manual contamination removal using the unscreened dataset. While there are no clear rules on the identification and removal of contamination (i.e. phage or horizontal gene transfer may be difficult to discriminate from contamination), we would like to provide some recommendations and guidance.

Because your target genome plus the contaminant sequences are essentially a small metagenome, and the tools useful for analyzing metagenomes are the ones we use for the contamination screening, you will initially log into the IMG/MER system and not IMG/ER.

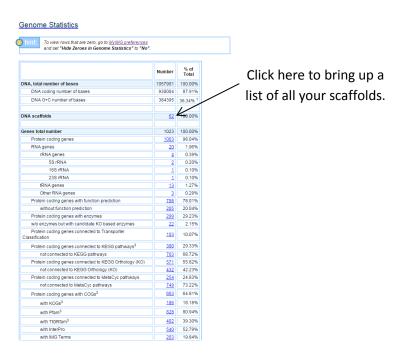
After logging into the IMG/MER system you need to find your genomes.

Under the Find Genomes tab you can search for any genome in IMG by a variety of criteria.

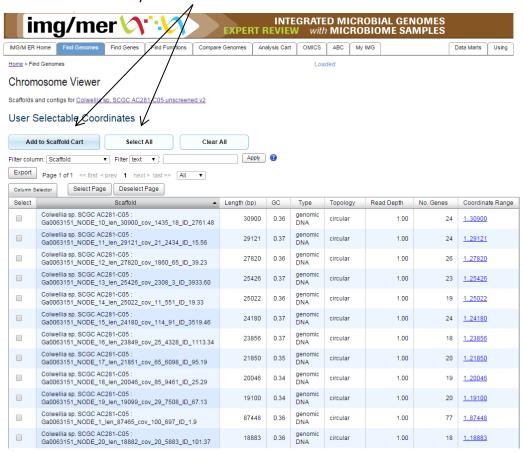


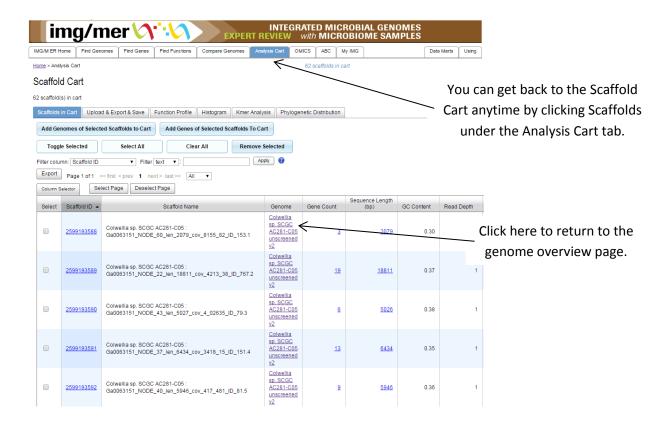


Each of your single cell genomes should have two versions in IMG: A "contamination screened" version that was decontaminated with JGI's automated pipeline, and an "unscreened" version that contains all the contigs greater than 2kb in length. Click on the name of the unscreened genome in order to bring up the genome overview page. Scroll down to the Genome Statistics.



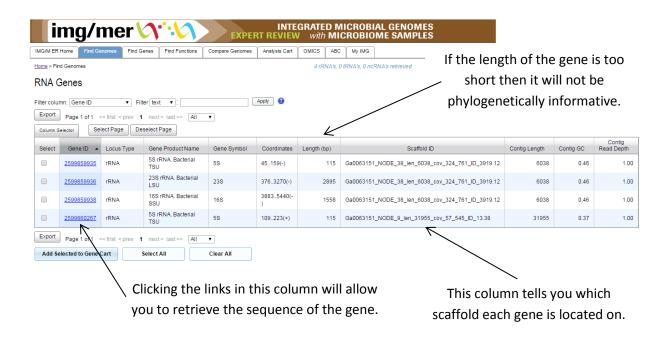
Select all the scaffolds and then add them to your Scaffold Cart.

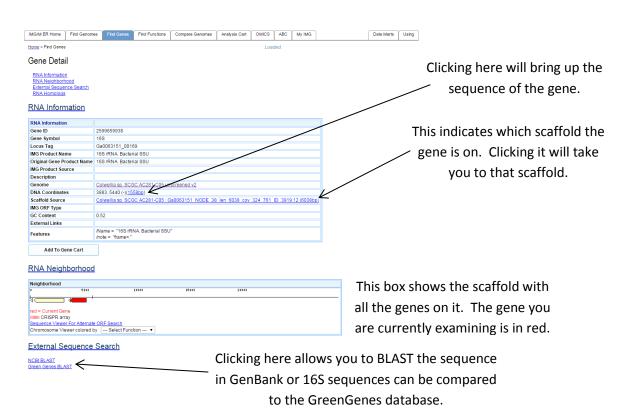




You will screen all of the scaffolds in your genome and put them in a clean data bin and a contaminants bin. A good place to start with contamination screening is to look at any ribosomal RNA sequences in your genome.

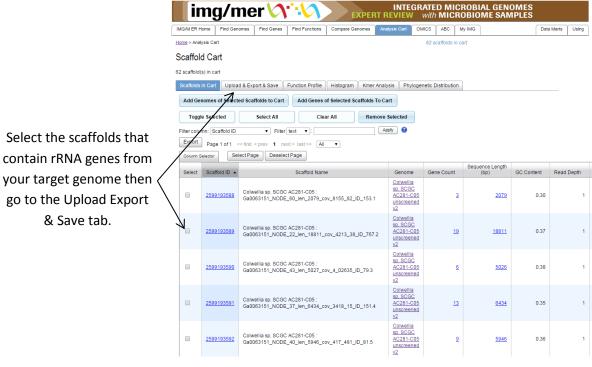




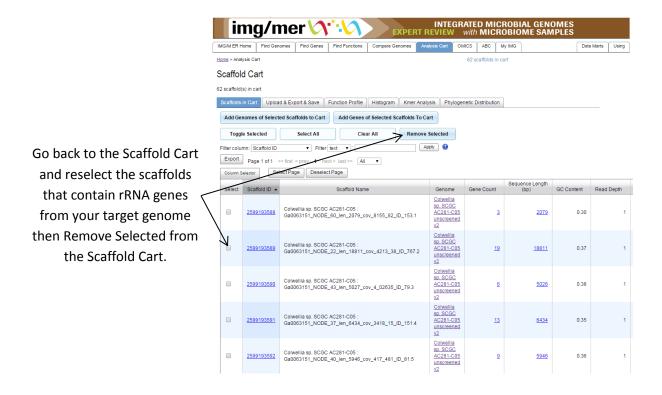


BLAST the rRNA sequences to see if they come from your target genome or from a contaminant.

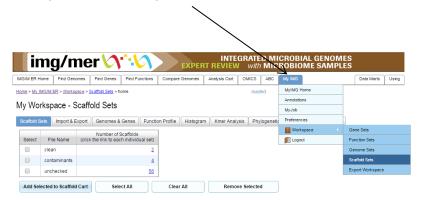
Return to the Scaffold Cart under the Analysis Cart tab.



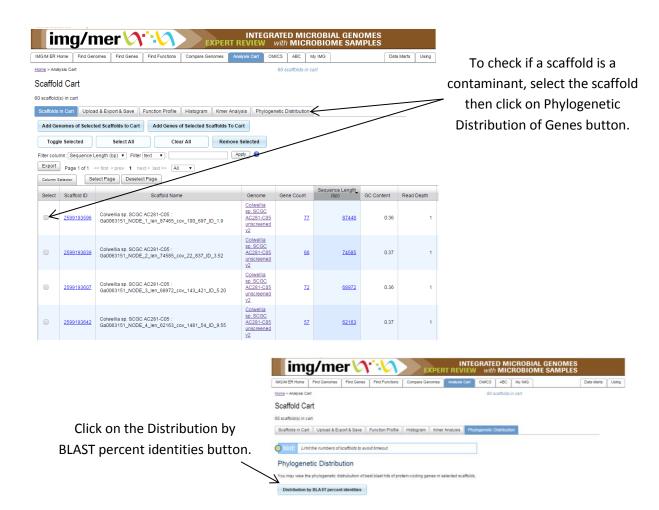




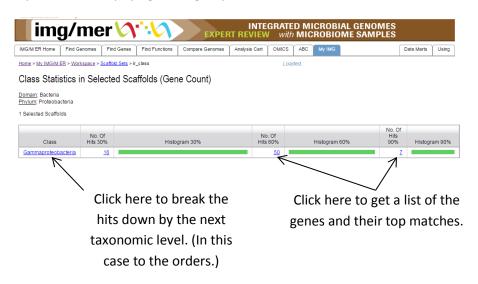
Follow the same procedure as above to select any scaffolds with contaminant rRNA genes, save them to a separate workspace and then remove them from the Scaffold Cart. At this time you may also want to save the remaining scaffolds in an "unchecked" workspace. When you leave IMG/MER the contents of your carts are not saved, but your workspaces are. You can get to the saved workspaces by going to Workspace under the My IMG tab.



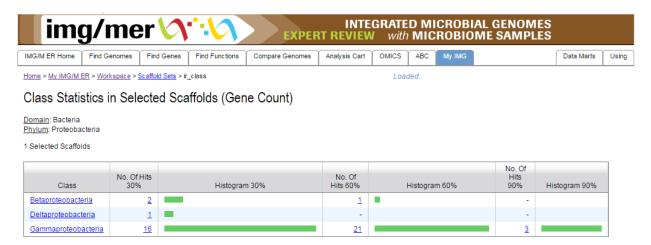
Go back to your Scaffold Cart that now only contains unchecked scaffolds. Since the rest of the scaffolds do not contain any rRNA genes to serve as phylogenetic markers, their identity must be determined by looking at all the genes present on each scaffold.



All of the genes in your genome have already undergone a BLASTx search. The phylogeny of the top hits for each gene in the scaffold is indicated. The scaffold shown below has 7 genes with a best hit of 90% identity or above to Gammaproteobacteria, 50 genes with a hit of 60%-90% to Gammaproteobacteria and 16 genes with top hits of 30%-60% to Gammaproteobacteria. In this case there are no genes with top hits to other phylogenetic groups.



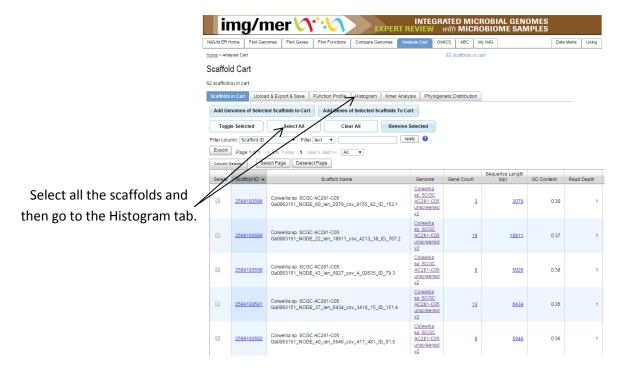
Due to gaps in the database as well as errors, you shouldn't place too much trust in a single gene's top hit. Instead, consider the consensus of all the genes on the scaffold before deciding whether it belongs to your genome or is a contaminant. For example, the scaffold below has three genes with best hits to Betaproteobacteria and one with a best hit to Deltaproteobacteria, but the majority of genes match Gammaproteobacteria and thus this scaffold can be confidently classified as coming from a Gammaproteobacterium.



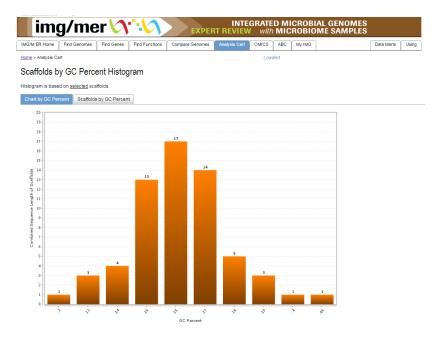
There are no strict rules about what should be removed as a contaminant. You will have to decide based on your data and on your own tolerance for mistakenly leaving a contaminant in or mistakenly throwing out something that really belongs.

Ideally every scaffold would be checked individually. However, we recognize that that could take a lot of time and may not be necessary. Instead, you can focus on the subset of scaffolds that are suspicious. There are three main methods for identifying suspect scaffolds that need to be checked: scaffold GC%, Kmer analysis, and the phylogenetic distribution of the genes in all your unchecked scaffolds.

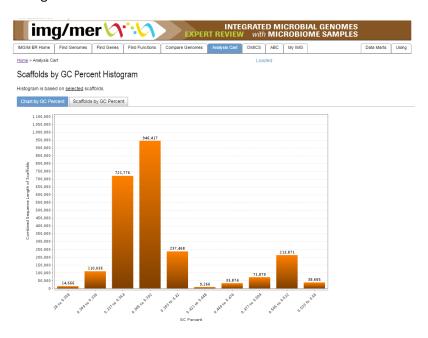
To identify scaffolds containing anomalous GC contents, go to the Scaffold Cart.



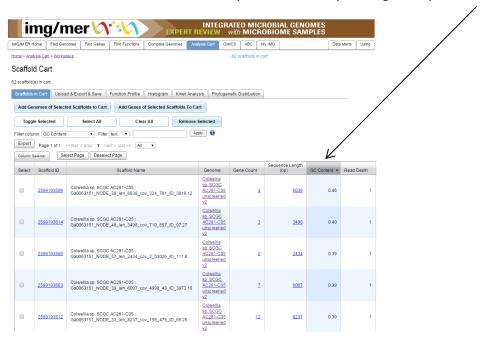




For a clean genome, you should have a single peak and all scaffolds should be within ~10% to either side of the center. Note that the histogram always gives you 10 bars/bins no matter how wide the spread in GC content is and the bins are not necessarily contiguous. For the above genome the bar to the far right (46% GC) is significantly higher than the next one (40%) and should be investigated. An example of a highly contaminated genome is shown below.

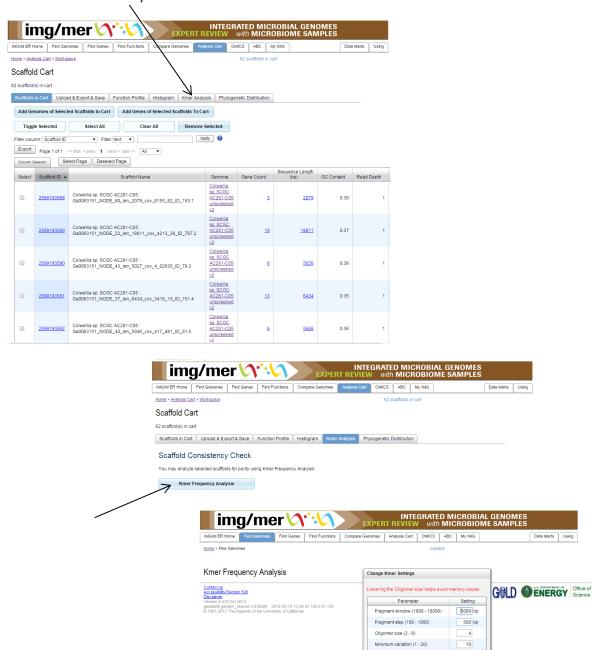


Go back to the Scaffold Cart and sort by Scaffold GC% by clicking the top of the column.



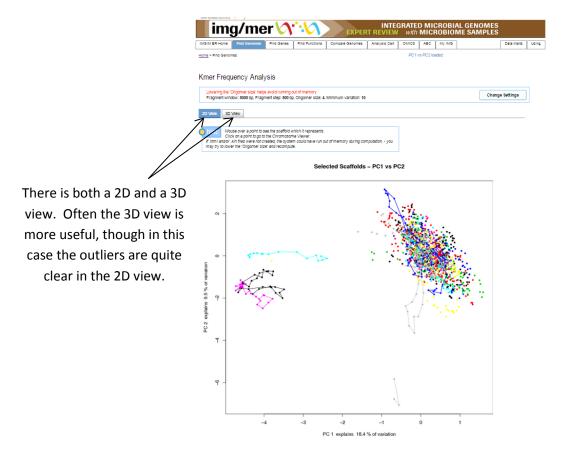
Now you can select the individual scaffolds with suspect GC% and check them using the Phylogenetic Distribution of Genes as described earlier. Bin these scaffolds to the clean or contaminant workspace and remove them from your Scaffold Cart.

To identify suspect scaffolds by Kmer analysis, go back to the Scaffold Cart, select all the scaffolds and click on the Kmer Analysis button.

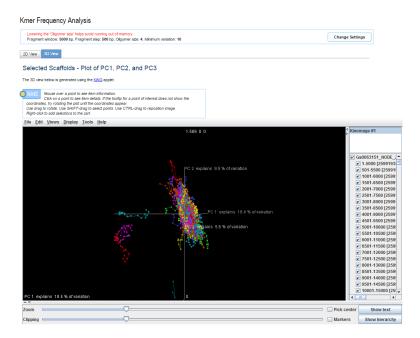


Begin by sticking with the defaults. The larger scaffolds have more statistical power, which will produce a more defined cloud of points. Also, it is easier to get a feel for the data with the few large scaffolds than if you included all of the data. Later you will want to rerun this analysis with a smaller fragment window to include all your scaffolds in the screen.

Clicking the Generate button will produce a Kmer plot.

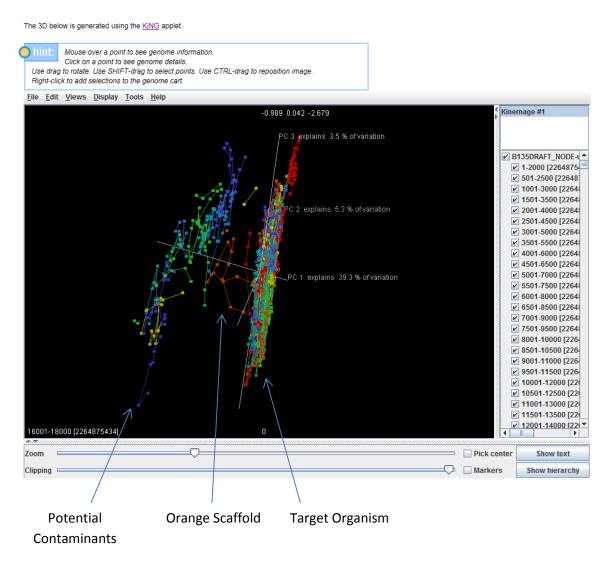


Click on the 3D View tab.

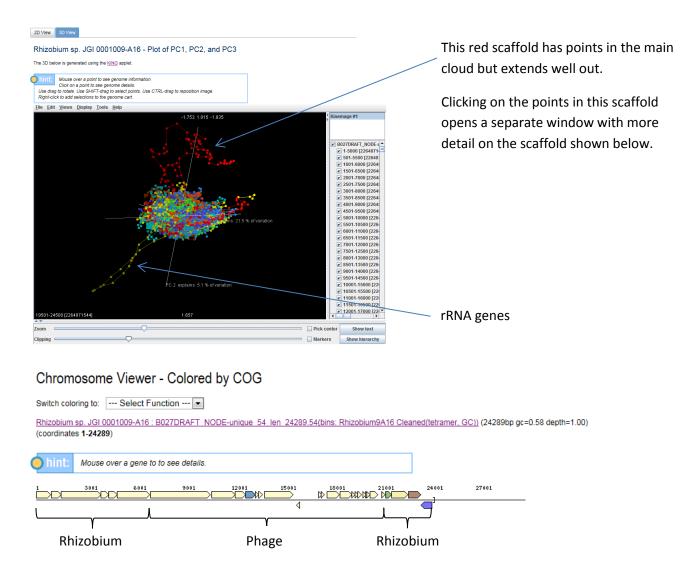


You can click and drag on the image to rotate it in three dimensions. First, look at the percent of variation explained by each principal component. If the percentages are all small (<~5%) then you have a very clean genome and the outliers are unlikely to be a problem.

Below is a fairly contaminated genome. Most points are in a large mass which is our target genome, but there is a distinct cloud of contaminant scaffolds to the left of the main cloud. By clicking on any of the points in the plot it will open a separate window of that scaffold.



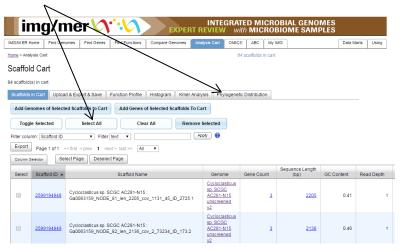
Note the Orange scaffold. This one starts in the main cloud, extends into the contaminant zone, and then returns to the main cloud. Upon examining this scaffold, we find that the region that extends out from the main cloud contains rRNA genes that match the target organism. Ribosomal RNA genes often contain a different GC content from the rest of the genome and thus will plot outside the main cloud of your target genome. Scaffolds that extend from the main genome cloud can also contain other interesting features.

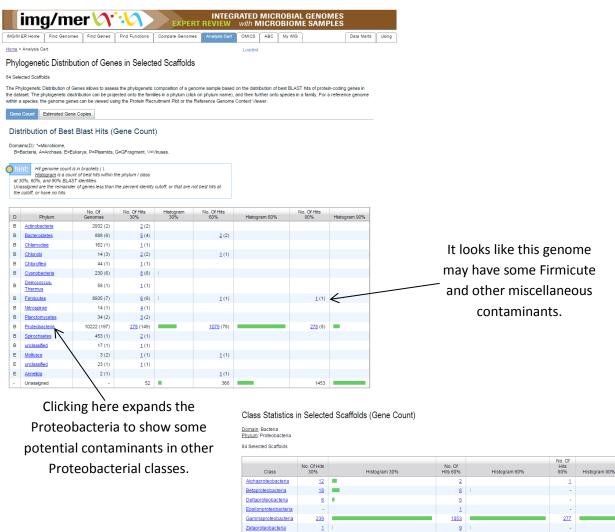


This scaffold is from a Rhizobium single cell and it contains 30 predicted genes. By doing a BLASTx search on each of the genes we found that the ends have high matches to proteins from various Rhizobium species. However, the genes in the middle, which caused the scaffold to stick out from the main cloud in the plot, have best matches to phage proteins. This cell appears to be infected with a lysogenic phage.

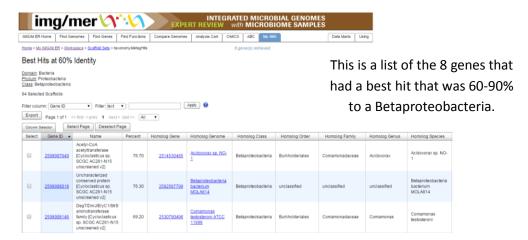
Suspicious scaffolds are those that lie outside the main cloud of points. You can identify these either by clicking on a point as above, or just by hovering the mouse over a point in the scaffold, the scaffold ID number appears in the bottom left corner of the plot.

Finally, you can identify suspicious scaffolds by looking at the Phylogenetic Distribution of Genes for all the unchecked scaffolds. Go back to your Scaffold Cart and Select All the scaffolds then click the Phylogenetic Distribution of Genes button.

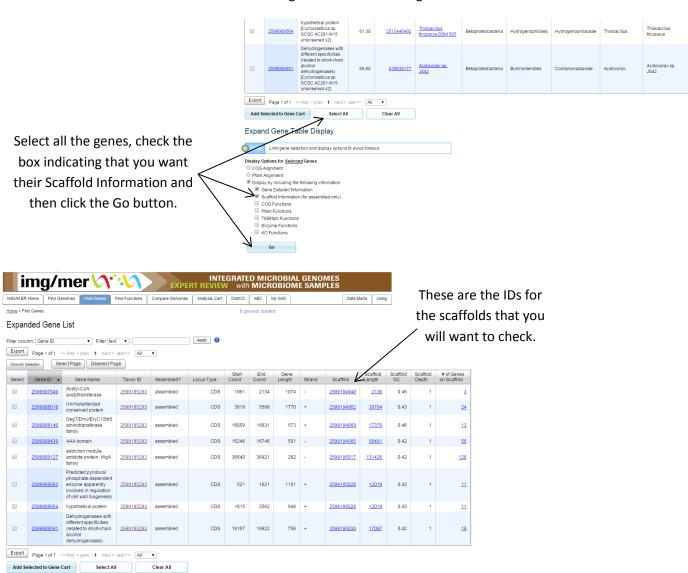




To identify which scaffolds contain these suspect genes, click on one of the numbers.

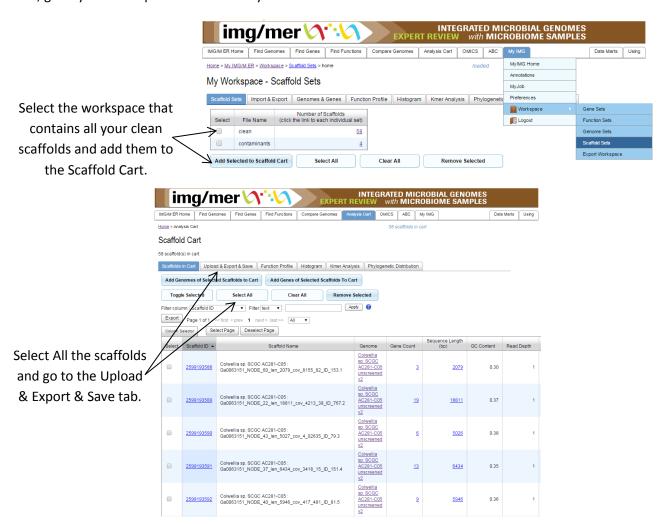


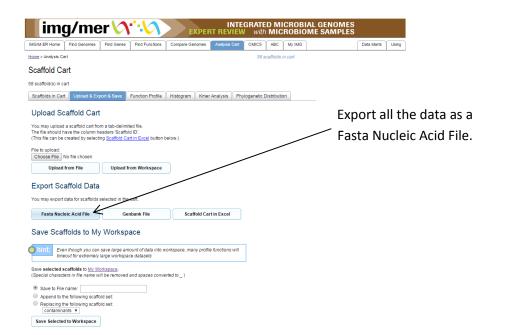
This list does not include the scaffolds that each gene is found on. To get that information scroll down.



Once you have finished cleaning up your genome you will want to upload it to IMG. The IMG system does not have a direct way to upload the data so first you must download a fasta file of your cleaned genome.

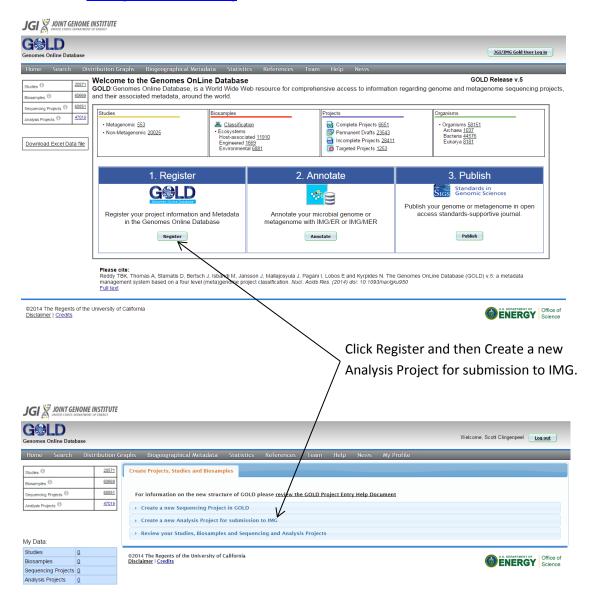
First, make sure your Scaffold Cart is empty by selecting any remaining scaffolds and removing them. Next, go to your workspaces under the My IMG tab.





Copy all the data and save it on your computer.

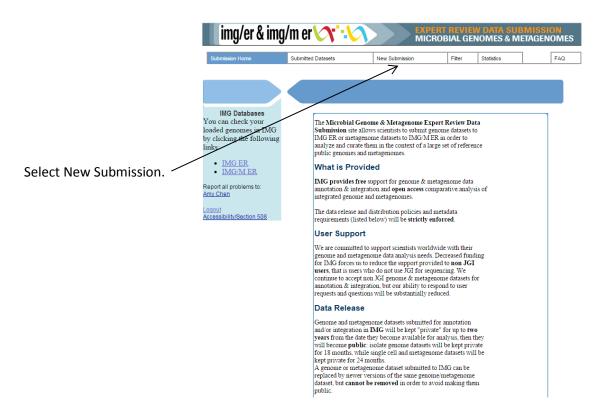
In order to upload this clean dataset to IMG you first need to get an Analysis Project ID from the GOLD website at www.genomesonline.org.



Once you have your Analysis Project ID, go to IMG to submit your dataset.



Select Submit Data Set from the Companion Systems tab from anywhere in IMG/MER, or click the Data Submission Site button on the home page.



Follow the instructions to upload your cleaned genome.

Congratulations! Once this is annotated and loaded into IMG you will have a single cell genome to analyze that is free of contamination sequences.

Author: Scott Clingenpeel March 2015